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RESULTS OF A FOURTEEN DAY ORAL-DOSING TOXICITY STUDY
OF AMMONIUM PERCHLORATE

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ABSTRACT

The use of ammonium perchlorate (AP), CAS registry number 7790-98-9, in the manufacture of solid rocket motors has resulted in soil and water contamination. Remediation levels proposed by the US EPA are extremely conservative because of an insufficient toxicity database (ECAO, 1992). Since the thyroid is the target organ and perchlorate inhibits iodine uptake by the thyroid, this 14-day study evaluated the potential of AP to produce changes in levels of triiodothyronine (T3), reverse T3 (rT3), T4, thyroid stimulating hormone (TSH), and thyroglobulin (Tg) by providing specific dose-response information for use in selecting doses for use in a 90-day subchronic toxicity study.

INTRODUCTION

This study was conducted to evaluate the potential of AP to produce alterations in thyroid function and to estimate the threshold dose for AP caused effects on thyroid hormone levels in rats. Results of this pilot study will enable selection of appropriate doses for a subchronic study that will evaluate the potential of ammonium perchlorate (AP) to produce alterations in paternal fertility, maternal pregnancy and lactation, growth and development of offspring of Sprague-Dawley rats. The animals received the test compound in drinking water as this route of treatment provides a more uniform dose than a single bolus dose produced by gavage and simulates the most probable route of human exposure in cases of environmental contamination. Blood levels of Tg, T_3, T_4, rT_3, and TSH were determined.

MECHANISM OF TOXICITY

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis and secretion of thyroid hormones. Perchlorate is known to competitively inhibit one of these steps, the iodine trapping mechanism, which incorporates free iodine into T_3 and T_4 (Capen, 1992) Low T_3 causes release of thyroid stimulating hormone (TSH) from the anterior pituitary, which results in thyroid follicular cell stimulation and hyperplasia. This hyperplasia may cause an increase in thyroid gland size (hypertrophy) (ORD, 1988).

The primary objective of this range-finder study was to determine toxicity information to establish doses of AP for a 90-day subchronic toxicity study. Sprague Dawley rats were exposed to varying concentrations of AP in their drinking water for a two-week period. The rats were then sacrificed and their thyroid hormone levels were measured by a radioimmunoassay technique. An additional goal of this work was to estimate a threshold dose based on changes from the control thyroid hormone data.

Data obtained from this study will be used to statistically estimate the threshold level for AP effects on the thyroid, the target organ for toxicity. The threshold level will be considered to be the "lowest observed adverse effect level" (LOAEL). The next lowest dose will be the "no observed adverse effect level" (NOAEL) and will be used for determination of the RfD for ammonium perchlorate using standard USEPA methodology. The dose-response data, reproductive and developmental toxicity information, and effects of AP on other organs, coupled with the establishment of a threshold dose for thyroid hormone effects will remove much of the uncertainty surrounding the provisional RfD and permit calculation of a RfD that should be at least an order of magnitude higher. Doses for the 90-day subchronic study will be based on results from this pilot study.

RELEVANCE OF RESEARCH

We wish to emphasize the need for this research by describing the lack of toxicity information, and specifically the lack of dose-response data for AP. Furthermore, it is intended to familiarize the reader with the mechanism by which thyroid hormone levels are inhibited by AP. Hormones are products of living cells that circulate in body fluids and produce a specific effect on the activity of cells remote from their point of origin. This inability of the thyroid gland to produce thyroid hormones, if not corrected, leads to excessive development of thyroid tissue cells (hypertrophy), an unusual increase in the number of tissue cells (hyperplasia) and the formation of abnormal masses of tissue (tumors that possess no physiologic function (neoplasia) in experimental animals as well as in humans (Capen, 1962; Hill et al 1989; Paynter et al., 1988).

The Thyroid Gland

This section explains the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated. Thyroxine (T4) and triiodothyronine (T3) are classically regarded as the two hormones produced by the thyroid gland. They contain 4 and 3 atoms of iodine, respectively, and are abbreviated as T4 and T3 according to their iodine content. These hormones are synthesized in the thyroid gland by iodinating thyroglobulin (Tg), an iodine containing protein stored in the thyroid (Goodman and van Middlesworth, 1980). This process can be divided into four steps. The first stage in the synthesis of thyroid hormones is the uptake of iodide from the blood by the thyroid gland. The trapped iodide is then combined with oxygen (oxidized). Once it is oxidized, iodine rapidly iodinates tyrosine residues within the Tg molecule to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) in a process is called iodide organification. Finally, either two DIT molecules or one DIT and one MIT molecule combine to form T4 and T3 respectively, in the ratio of 5:1, so that most of the hormone released is T4.

Under normal conditions the thyroid may concentrate iodide up to 25 times higher than the blood concentration. This ratio may be considerably higher (250:1) when the thyroid is active. Iodide uptake may be blocked by several anions, one of which is perchlorate (Goodman and van Middlesworth, 1980).

T4 is the major hormone secreted from the thyroid and is converted to more active T3 in a variety of peripheral tissues, including the pituitary gland. T4 is also metabolized to rT3 which is hormonally inactive and has no know function, except perhaps as an inhibitor of the conversion of T4 to T3 (Hill et al., 1989; Stevens, 1985, Goodman and van Middlesworth, 1980).

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T4 and T3. The mechanism involves the anterior pituitary of the brain (Hill et al., 1989; Paynter et al., 1988; Houk, 1980). Thyroid-stimulating hormone (TSH, or thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to create new thyroid hormones, is very important in the feedback mechanism. It independently promotes iodide trapping and iodination of Tg. The rate of release of TSH from the pituitary is controlled by the circulating levels of T4 and T3.

If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased. If exogenous thyroid hormone is administered, eventually the thyroid gland becomes inactive and atrophied. The blood concentrations of both T4 and T3 are important factors in the release of TSH (Capen, 1992; Hill et al., 1989; Paynter et al., 1988; Goodman and van Middlesworth, 1980).

According to Goodman and van Middlesworth (1980), an exact description of the role of the thyroid hormones is not yet possible. However, they discuss several studies which indicate that if the hormones are not present in the early stages of life, mental maturation, bone development, and the central nervous system are negatively affected. In some instances, the lack of bone development can be corrected by administering T4. However, administration of even tremendous amount of T4 does nothing to correct

mental retardation, suggesting that the hormones must be present during critical periods in order for normal development to occur.

Thyroid Gland Neoplasia

Hill et al explains that thyroid neoplasia may be induced by exposure of experimental animals to a variety of treatment regiments, chemicals produced outside the body (exogenous), or physical agents. "It has been recognized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dysfunction, in particular, [enlargement of the thyroid gland and increased metabolic rate] hypothyroidism." Factors inducing hypothyroidism include iodine deficiency, surgically removing part of the thyroid gland, and the transplantation of TSH-secreting pituitary tumors. "The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH." Whatever the cause (i.e. low iodine diet, blocked iodide uptake by an anion), prolonged stimulation of the thyroid-pituitary feedback mechanism that results in the release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia. However, thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone or by surgically removing the pituitary gland (hypophysectomy) (Hill et al., 1989).

A recent review of chemical injury of the thyroid (Capen, 1992) showed that rodents treated with agents that directly interfere with thyroid hormone production in the thyroid gland depress T3 and T4 levels resulting in a compensatory increase of TSH. This TSH stimulation of the thyroid gland leads to hypertrophy, hyperplasia, and neoplasia in rodents. In addition, this excessive secretion of TSH alone, without any chemical exposure, produces a high incidence of thyroid tumors in rodents. Capen concluded that thresholds for agents which inhibit iodide uptake by the thyroid can be established by determining the dose that fails to elicit an elevation in the circulating level of TSH. Hence, the threshold concentration of perchlorate, i.e., the perchlorate concentration below which there is no depression of T3 and T4 accompanied by TSH elevation, is completely protective against carcinogenesis.

Human Data.

Brabant et al conducted a study in which 5 healthy males were exposed to an oral treatment of 300 mg of perchlorate 3 times daily over a 4-week period. Mean serum TSH levels decreased slightly and the thyroid volumes were unaltered. The body weights of the volunteers were not provided. However, using the standard 70 kg default body weight used for risk assessment results in a dose of 12.86 mg/kg/day. This would suggest that the threshold dose for thyroid hormone effects in healthy humans is higher than 12.86 mg/kg/day.

Burgi et al (1974) administered 200 mg of perchlorate 3 times daily to three healthy females and two healthy males for 8 days. The average dose for the females was 11.04 mg/kg/day and the average for the males was 8.22 mg/kg/day. These doses were sufficient to completely block iodide uptake by the thyroid as measured in the urine. However, thyroid hormone levels were not measured in order to determine if this dose produced a decrease in T3 and T4 or an increase in TSH levels.

A study that used potassium perchlorate (KP) to displace iodide from the thyroid gland (Stanbury and Wyngaarden, 1952) was used as the basis for deriving the EPA's provisional RfD. Standbury and Wyngaarden found that 0.14 mg/kg/day (assuming a body weight of 70 kg) was not sufficient to completely block iodide uptake. However, 1.4 mg/kg/day was sufficient to block 90% of the measured iodide. The study did not evaluate the effects of KP on thyroid hormone levels and only three doses were given. These three studies are summarized in Table 1.

Study	Exposure Conditions	Conclusions
Brabant et al	-12.88 mg/kg/day	-TSH levels decreased slightly
Burgi et al	-11.04 mg/kg/day for four weeks (males) -8.22 mg/kg/day for four weeks (females)	-Sufficient to completely block iodide uptake by the thyroid
Stanbury and Wyngaarden	-0.14 mg/kg (once) -1.4 mg/kg (once)	-55% of initially accumulated radioactive iodide was present in the neck -15% of initially accumulated radioactive iodide was present in the neck

Table 1. Summary of human studies using perchlorates.

Animal Data.

   With regard to animal data, Shigan conducted a study in which 'white rats' were given AP under various conditions (1963, translated from Russian, 1994). The rats were treated with doses ranging from 2500 to 8500 mg/kg and observed for 15 days. Even though most of the animals died during the first 3 days, Shigan was able to calculate the dose of AP which killed fifty percent of the total experimental population (i.e., the LD_{50}) (see Table 2). 'White rats' were also exposed to AP under two other conditions described in Table 2. However, these results do not provide any insight in determining a threshold dose because there were no doses given at low concentrations.

Exposure Conditions	Conclusions
4200 mg/kg (once)	-LD_{50} value
650 mg/kg/day for one month	-No noticeable cumulative properties
190 mg/kg/day for three months	-Affects the regulation of the involuntary nervous system -Causes a statistically reliable change in the protein fractions of the blood serum -Disrupts the liver's ability to produce glycogen for carbohydrate storage

Table 2. Results of Shigan's Experiments on 'white rats'

   Mannisto et al (1979) studied the effects of Potassium Perchlorate (KP) on the thyroid of the Sprague-Dawley rat. He found that doses of KP from 7.6 to 15.3 mg/kg/day administered over a 4 day period reduced serum T3 and T4 levels and increased TSH levels.

   These animal experiments do not provide enough information on which to base an accurate RfD since dose-response data on thyroid hormone levels are lacking (e.g., Shigan) or the period of

administration was too short (e.g., Mannisto). Since the EPA based their provisional perchlorate RfD on the Stanbury and Wyngaarden study and predicted that chronic administration of perchlorate at the dose used in that study would likely have resulted in lowering of the patients T3 and T4 levels, with subsequent increases in the levels of TSH, this hypothesis was tested by designing a study in which changes in thyroid hormone levels in the Sprague-Dawley rat would be measured in response to increasing doses of AP (Caldwell and Mattie, 1995). A 14-day pilot study provided specific dose-response data over a wide range of doses, from which a threshold level for thyroid hormone effects of AP could be estimated (Caldwell et al, 1995). Since increased levels of TSH are a sign that the thyroid has been disturbed, if a dose of perchlorate can be found from which there is no observed statistically significant increase in the amount of TSH in the blood, this dose can be considered at or below the threshold dose (Capen, 1992). Subsequently, this dose can be used in deriving a RfD.

PILOT STUDY

Groups of six male and six female Sprague-Dawley rats were dosed with AP in drinking water at concentrations of 0 (control), 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L. Animals were sacrificed after fourteen days and thyroid hormone levels were measured using a radioimmune assay technique. The actual dose of AP administered to each animal was calculated by multiplying the concentration of AP administered in the drinking water by each animal's average water consumption over the 14-day period and dividing this number by each animal's average body weight over the 14-day period. Selected thyroid hormone data are presented in Table 3.

RESULTS

General

AP did not have a statistically significant effect on the average water consumption of either sex at the concentrations administered. Nor did AP have a statistically significant effect on the body weight gain of either sex; both sexes gained weight in the same manner over the two-week period.

Thyroid hormone levels

AP had a statistically significant affect on the thyroid hormone levels in both sexes. The T3 and T4 levels decreased while TSH, rT3, and Tg increased with increasing doses of AP in both males and females; however, the sexes were not affected to the same extent.

Dose (mg/kg-d) (male,female)	T3-Male	T3-Female	T4-Male	T4-Female	TSH-Male	TSH-Female
0 (control)	133	129	5.1	5.0	14.5	11.3
0.11, 0.12	124	85	4.8	4.4	15.0	13.1
0.44, 0.47	106	84	4.7	4.1	16.9	14.6
1.11, 1.23	90	81	4.3	4.0	20.2	15.4
2.26, 3.06	76	79	4.2	3.9	30.2	17.4
4.32, 4.91	71	72	4.1	3.7	31.2	19.2
11.44, 11.47	66	69	3.4	3.3	34.0	22.7
22.16, 24.66	66	66	3.0	2.9	37.4	29.9

Table 3. Effects on Thyroid Hormone Levels.

CONCLUSIONS

The data derived from the two-week study show a decrease of T3 and T4 with a concomitant rise in TSH with increasing doses of AP. The NOAEL was 0.44 mg/kg/d and LOAEL was 1.11 mg/kg/d for the male rats and 0.12 and 0.47 mg/kg/d, respectively, for female rats. This is consistent with the literature which shows a higher level of circulating TSH in male rats compared to females (Jubb et al, 1993).

Long-term perturbations of the pituitary-thyroid axis are more likely to predispose laboratory animals to a higher incidence of proliferative lesions than is the case in the human thyroid. This appears to be particularly true in the male rat in which there usually are higher circulating levels of TSH than in females. The greater sensitivity of the animal thyroid to derangement by chemicals and physiologic perturbations also is related to the shorter plasma half-life of T4 (12-24 hours) than in humans (5-9 days), due, in part, to the considerable differences between species in the transport proteins for T4. In humans, circulating T4 is bound primarily to thyroxine-binding globulin (TBG), but this high-affinity binding protein is not present in rodents. T3 is transported bound to TBG and albumin in humans, but only to albumin in rats. In general T3 is bound less avidly to transport proteins than is T4, resulting in a faster turnover and shorter plasma half-life in most species (Jubb et al, 1993).

Although the data established the NOAEL for thyroid hormone effects, more research is needed in order to determine a precisely defined threshold dose for such effects, and to evaluate the dose-response relationship for other potential toxic effects of AP, such as changes in the production of red blood cells (i.e., hematopoiesis). A planned 90-day study will use doses selected to better estimate the threshold dose and to refine the NOAEL for thyroid hormone effects and other toxicity endpoints. The additional data obtained from this subchronic study will support the use of a less conservative uncertainty factor since data gaps on reproductive and developmental toxicity will be filled, the threshold for effects on thyroid hormone homeostatis will be determined, and investigation of hematopoietic effects will be performed. Therefore, a RfD between 1E-3 mg/kg/day and 1E-2 mg/kg/day appears to be reasonably obtainable.

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